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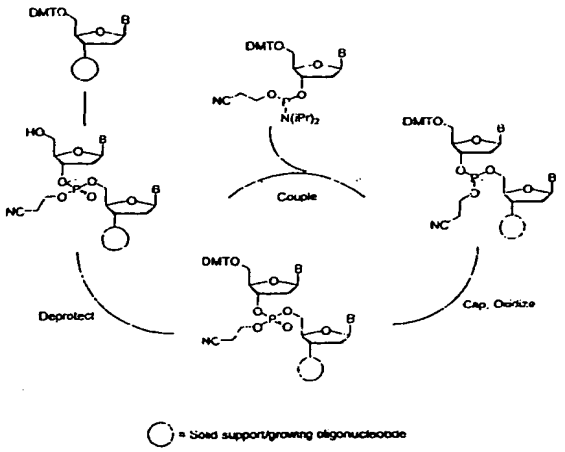
(54) Synthesis of oligonucleotides

(57) The invention provides a method for synthesizing oligonucleotides using carbonate protection of hydroxyl groups and nucleophilic deprotection reagents. The deprotection reagents irreversibly cleave the carbonate protecting groups while simultaneously oxidizing the internucleotide phosphite triester linkage, and can be used in aqueous solution at neutral to mildly basic pH. The method eliminates the need for separate deprotection and oxidation steps, and, since the use of acid to remove protecting groups is unnecessary, acid-induced depurination is avoided. Fluorescent or other readily detectable carbonate protecting groups can be used, enabling monitoring of individual reaction steps during oligonucleotide synthesis. The invention is particularly useful in the highly parallel, microscale synthesis of oligonucleotides. Reagents and kits for carrying out the aforementioned method are provided as well.

Figure 1

3'-5' Synthesis With DMT Protection



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INCOMPLETE SEARCH SHEET C

Application Number
EP 99 30 6168

Claim(s) searched completely:
3

Claim(s) searched incompletely:
1,2,4-10

Reason for the limitation of the search:

Present claims 1,2,4-7,10 relate to a method containing a reagent defined by reference to a desirable characteristic or property, namely a deprotecting reagent effective to convert the carbonate-protected hydroxyl group to a free hydroxyl moiety and simultaneously oxidize the phosphite triester linkage to give a phoshotriester linkage.

The claims cover all methods containing this reagent having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for only a very limited number of such reagents. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the reagent by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed. Thus the above claims have only been completely searched in the light of claim 3 i.e. where the reagent is a peroxide.

Furthermore with regard to claims 8 and 9, the initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it is impossible to determine which parts of the claims may be said to define subject-matter for which protection might legitimately be sought (Article 84 EPC). For these reasons, a meaningful search over the whole breadth of the claim(s) is impossible. Consequently, the search has been restricted to:

compounds given in the Formula of claim 8, where R3 = aryloxycarbonyl.

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 99 30 6168

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	MCGALL, G H.ET AL: "THE EFFICIENCY OF LIGHT-DIRECTED SYNTHESIS OF DNA ARRAYS ON GLASS SUBSTRATES" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY,US,AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, vol. 119, no. 22, 4 June 1997 (1997-06-04), pages 5081-5090, XP000775689 ISSN: 0002-7863 * page 5082, scheme 1, compounds 7a - 7k * ---	8,9	
X	PIRRUNG ET AL.: "COMPARISON OF METHODS FOR PHOTOCHEMICAL PHOSPHORAMIDITE-BASED DNA SYNTHESIS" JOURNAL OF ORGANIC CHEMISTRY, vol. 60, 1995, pages 6270-6276, XP002088249 * COMPOUNDS 1-4,6, PAGE 6274, COLUMN 1 * ---	8,9	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
X	DOUGAN ET AL.: "SYNTHESIS AND RADIOIODINATION OF A STANNYL OLIGODEOXYRIBONUCLEOTIDE" NUCLEIC ACID RESEARCH, vol. 25, no. 14, 1997, pages 2897-2901, XP002167902 * compound 5, page 2899, scheme 1; page 2898, 2nd paragraph, lines 9-11 * ---	8,9	
D,X	IWAI ET AL.: "5'-LEVULINYL AND 2'-TETRAHYDROFURANYL PROTECTION FOR THE SYNTHESIS OF OLIGORIBONUCLEOTIDES BY THE PHOSPHORAMIDITE APPROACH" NUCLEIC ACID RESEARCH, vol. 16, no. 20, 1988, pages 9443-9456, XP002167839 * page 9446, table 1 * ---	10	
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EPO FORM 1503 03.82 (P04C10)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 99 30 6168

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
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28-05-2001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9628457 A	19-09-1996	US 5908926 A	01-06-1999
		AU 5422896 A	02-10-1996
		CA 2215657 A	19-09-1996
		EP 0815114 A	07-01-1998
		JP 11501936 T	16-02-1999
EP 0742287 A	13-11-1996	US 6156501 A	05-12-2000
WO 9839348 A	11-09-1998	US 6147205 A	14-11-2000
WO 9954509 A	28-10-1999	AU 3659199 A	08-11-1999
		EP 1071821 A	31-01-2001
WO 0061594 A	19-10-2000	DE 19915867 A	19-10-2000
		AU 5059800 A	14-11-2000
WO 0018778 A	06-04-2000	AU 6270499 A	17-04-2000
US 6022963 A	08-02-2000	US 6147205 A	14-11-2000